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Germination of the invasive legume *Lupinus polyphyllus* depends on cutting date and seed morphology

Yves P. Klinger[1], Rolf Lutz Eckstein[2], David Horlemann[1], Annette Otte[1], Kristin Ludewig[1]

1 *Division of Landscape Ecology and Landscape Planning, Research Centre for Biosystems, Land Use and Nutrition (iFZ), Justus Liebig University Giessen, Heinrich-Buff-Ring 26–32, D-35392 Giessen, Germany*
2 *Department of Environmental and Life Sciences, Biology, Karlstad University, SE- 651 88 Karlstad, Sweden*

Corresponding author: Yves Klinger (yves.p.klinger@umwelt.uni-giessen.de)

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Abstract

In semi-natural grasslands, mowing leads to the dispersal of species that have viable seeds at the right time. For invasive plant species in grasslands, dispersal by mowing should be avoided, and information on the effect of cutting date on the germination of invasive species is needed. We investigated the germination of seeds of the invasive legume *Lupinus polyphyllus* Lindl. depending on the cutting date. We measured seed traits associated with successful germination that can be assessed by managers for an improved timing of control measures. To this end, we sampled seeds of *L. polyphyllus* on six cutting dates and analyzed the germination of these seeds in climate chambers and under ambient weather conditions. We collected information on seed morphology (color/size/hardseededness) for each cutting date to identify seed traits associated with successful germination. Observed germination patterns were highly asynchronous and differed between seeds cut at different dates. Seeds cut early, being green and soft, tended to germinate in autumn. Seeds cut late, being dark and hard, were more prone to germinate the following spring, after winter stratification. This allows the species to utilize germination niches throughout the year, thus indicating a bet-hedging strategy. Seed color and the percentage of hard seeds were good predictors of germination percentage, but not of mean germination time and synchrony. Managers should prevent the species producing black and hard seeds, while cutting plants carrying green and soft seeds is less problematic. Furthermore, germination patterns differed between climate chambers and the common garden, mainly because germination of dormant seeds was lower in climate chambers. More germination experiments under ambient weather conditions should be carried out, as they can give information on the germination dynamics of invasive species.

Keywords

dormancy, grassland management, lupine, phenology, seed traits

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Introduction

The timing of germination determines which environmental conditions the seedling will experience and thereby influences a variety of plant characteristics (Casas et al. 2012). Consequently, the germination ecology of a species largely decides in which habitats and under which climates it may establish. The introduction of species to new ranges often leads to new germination conditions (Kudoh et al. 2007), and the ability to germinate successfully under a variety of environmental conditions is a characteristic of many successful and widespread invasive species (Baker 1974; Wainwright and Cleland 2013). Whether seeds are viable depends largely on their development stage, which is influenced by the timing of seed set and seed ripening.

In semi-natural grasslands, the mowing date is the environmental factor that most strongly determines the timing of seed release. Furthermore, mowing is a way of seed dispersal for species that have viable seeds at the right time. In most cases, the dispersal of mature seeds after mowing is a desirable process, as it is responsible for sustaining a high plant diversity in semi-natural grasslands (Auffret 2011; Humbert et al. 2012). In other cases, such as weeds or non-native invasive species, dispersal of ripe seeds by mowing is not wanted (Wilson et al. 2009) and shifts in grassland management and the time of cutting may create opportunities for invasives to establish in these ecosystems. Consequently, understanding the germination ecology of invasive plants is essential for their management and control and for limiting their spread to new sites.

Established invasive species are often more challenging to manage than newly arrived species (Simberloff 2003). Ideally, control measures would take place before seed formation, but time windows for adequate management can be short in areas where different conservation goals have to be matched. In the case of species invading mountain grasslands, e.g., mowing of areas critical for the protection of ground-nesting birds has to be postponed until nesting is finished, which means that invasive species may have produced viable seeds by the time of mowing. Consequently, managers are looking for information on the relationships between cutting dates, seed morphology, and seed germinability. *Lupinus polyphyllus* Lindl. is a widespread perennial legume originating from North-America. It is widely found as an ornamental plant (Fremstad 2010) and commonly used for soil stabilization and soil melioration (Rehfuess et al. 1991). Due to its many uses, the species is naturalized in different regions all over the world, e.g. in Europe (Fremstad 2010; Hejda 2013), New Zealand (Holdaway and Sparrow 2006) and Chile (Meier et al. 2013). Invaded habitats include road verges (Valtonen et al. 2006), riparian terraces (Meier et al. 2013), and mountain grasslands (Klinger et al. 2019). Due to its ability to fix nitrogen, it is considered an ecosystem engineer and may cause unwanted ecosystem effects (Hiltbrunner et al. 2014). In invaded habitats, *L. polyphyllus* is capable of overgrowing and shading the underlying vegetation and may cause a considerable decline in the richness of small species (Thiele et al. 2010; Hiltbrunner et al. 2014), while promoting the spread of tall-growing, nitrogen-demanding vegetation (Otte and Maul 2005). Meadows invaded by this species provide hay of low fodder quality, because of its high water-content and the presence of alkaloids in *L. polyphyllus* (Hensgen and Wachendorf 2016).

Despite the importance of seed ecology for the spread and establishment of species, there is often insufficient knowledge concerning germination and ripening characteristics of invasive species (Gallinat et al. 2018). The capability of seeds to after-ripe and germinate, which depends on the interaction between phenology and cutting date, may have important implications for the management of invasive species in grasslands. Therefore, we investigated the germination of the invasive legume *L. polyphyllus* in relation to the cutting date. Over the course of the vegetation period, i.e., weekly from the beginning to the end of fruiting, we sampled seeds from five locations invaded by *L. polyphyllus*. We combined two experiments to investigate the germination of *L. polyphyllus*: A common garden experiment to analyze the germination patterns under ambient weather conditions and a climate chamber experiment under standardized conditions. We aim to provide management recommendations based on seed traits such as seed color and hardseededness that may help to decide when fruiting lupine stands should be cut and when plant material has to be removed from the sites after mowing.

Specifically, our research hypotheses were:

1) The germination ability of *L. polyphyllus* seeds increases with later cutting date. Consequently, we expect a higher germination percentage, a shorter mean germination time, and a higher synchrony of germination with later cutting date.
2) Seed traits such as seed size, seed color, and the percentage of hard seeds provide reliable information about the germination ability of seeds sampled at different dates. We expect larger seeds, seeds with darker color and harder seeds to show higher germination percentage, shorter mean germination time and higher synchrony compared to small, green, and soft seeds.

Methods

Seed sampling, seed handling, and experimental design

Seeds were collected in the Rhön UNESCO Biosphere Reserve, in central Germany. The study area (from 50°26'N to 50°32'N and from 09°54'E to 10°05'E), a part of the Biosphere Reserve, is situated between 600 m and 950 m a.s.l. It is characterized by large and coherent semi-natural grasslands of high conservational value that are non-intensively used as meadows and pastures (e.g., Habitats Directive 92/43/EEC, habitat types 6520: mountain hay meadows, and 6230: species-rich *Nardus* grasslands). These grasslands have a centuries-long land-use history of mowing and pasturing with low nitrogen-inputs. In the 1990s, the traditional mowing date in early July was postponed to August and September, in order to safeguard the populations of protected ground-nesting birds and because the meadows decreased in importance for local farmers. This allowed *L. polyphyllus*, already present along roadsides in the area, to produce seeds before mowing and to spread extensively into the meadows. During the past 20 years, parts of the region were heavily invaded, with the area covered by *L. polyphyllus* dou-

bling in some localities (Klinger et al. 2019). This invasion is considered a major threat to the biodiversity of the mountain grasslands in the study region. Depending on site conditions, *L. polyphyllus* can reach a height of 60 to 150 cm. In June and July inflorescences are formed, each consisting of 50 to 80 single flowers (Fremstad 2010; Bundesamt für Naturschutz 2017). *L. polyphyllus* develops seed pods with four to twelve seeds, which burst at seed maturity and spread the seeds ballistically up to several meters (Otte et al. 2002; Volz 2003). Per plant, up to 2500 seeds can be produced (Aniszewski 2001).

Seeds of *L. polyphyllus* were manually collected from five meadows (*sampling locations*) over six weeks (July–August 2015; *cutting dates*). The distance between sampling locations ranged between 1500 and 5000 meters. For each cutting date and location, we sampled one inflorescence each from ten plants for the germination experiments. From each inflorescence, we randomly took one pod and determined seed size, seed color, and the proportion of hard seeds. For seed color, we distinguished between four colors: green, dark green, brown and black. Seeds with different pigmentations and puncturing (see Aniszewski 2001) were integrated to the different classes according to the predominant color, seeds were assigned the color "black" when they were considerably darker than brown seeds. Usually, seeds of several colors were found on the same location or even within the same seed pod. To determine the average color for each replicate, we gave ranks from one (green) to four (black) to each color and calculated the median. For seed hardness, we classified the seeds into five classes, from undeveloped and very soft to very hard. Based on these data, we calculated mean seed size, average seed color and the proportion of hard seeds for each replicate. For the germination experiments, we pooled the seeds within each *sampling location.* Seeds were manually cleaned, air-dried and stored in darkness at room temperature (app. 20 °C) until the start of the germination experiments on September 28th, 2015.

Laboratory experiments are a standardized tool to investigate germination in a controlled environment and can provide information on germination cues, dormancy, and other factors (Baskin and Baskin 2014). Nonetheless, germination in the laboratory often differs from germination under (semi-)natural conditions (Grime et al. 1981; Hölzel and Otte 2004) and thus gives only a limited representation of germination patterns that can be observed in the field. We combined a climate chamber experiment and a common garden experiment to study the germination of *L. polyphyllus* both under standardized and ambient weather conditions. A factorial experimental design was used to analyze the effects of *cutting date* (6 dates), *sampling location* (5 locations), and *temperature* (day/night: 20/10 °C and 15/5 °C; only in the climate chamber experiment) on seedling emergence. Germination was defined as protrusion of the radicle.

In the climate chamber experiment (from September 28th, 2015 to July 28th, 2016), seeds were placed into petri dishes with distilled water (25 seeds per replicate) in climate chambers (Rumed type 3401, Rubarth Apparate GmbH). Each treatment combination (*cutting date* × *sampling location* × *temperature*) was replicated five times, resulting in 300 petri dishes. For incubation in climate chambers, we exposed the seeds to 12 h light and 12 h darkness and two diurnally fluctuating temperatures (15/5 °C and 20/10 °C) that represent spring and early summer temperature conditions. Similar fluctuating temperature conditions have been applied by Elliott et al. (2011). Moisture

content of the Petri dish was controlled during the experiment. For seeds in the climate chambers, germination was checked once a week and seedlings were removed.

In the common garden experiment, germination was observed under ambient weather conditions from September 17th, 2015 to July 14th, 2016. The seeds were placed on a 1:1 mixture of sand and commercial potting soil (Fruhstorfer Erde, Type P, Industrie-Erdenwerke Archut GmbH, Lauterbach/Germany) in trays (18 × 28 cm) in a common garden at the research station Linden-Leihgestern of the Justus-Liebig University (50°32'N, 8°41'E). Per tray, 25 seeds were used (n = 5 for each *cutting date* × *sampling location* combination, resulting in 150 trays). Seeds were protected from predation using wire cages. For seeds in the common garden, germination was checked once every seven to fourteen days. After three months of incubation, germination decreased in both experiments and thus was checked every other week. After ten months of incubation, the experiments ended since no further germination was observed. By the end of the experiments, the remaining seeds were covered by mold and collapsed when pinched by hand. Thus, the remaining seeds were considered dead (following Baskin and Baskin 2014).

Germination variables and statistical analyses

As response variables, we calculated the germination percentage (%), mean germination time (days) and synchrony of germination (unitless) per replicate (according to Ranal and Santana 2006; Ranal et al. 2009). The germination percentage is the proportion of germinated seeds of the total number of seeds. Mean germination time and synchrony of germination were calculated based on seedling counts over time (Ranal et al. 2009). Mean germination time is a measurement of the weighted average time required for germination (Ranal and Santana 2006). The synchrony index is a measure for the overlapping of germination that ranges from 0 (when no two seeds germinated at the same time) to 1 (when all germinating seeds germinated at the same time; for details see Ranal et al. 2009).

Seeds from the climate chamber experiment and from the common garden experiment were analyzed separately. The effects of the experimental variables *cutting date*, *sampling location* and *temperature* on the response variables germination percentage and germination time were analyzed using linear mixed-effect models (LMM) and synchrony of germination using generalized linear mixed-effect models (GLMM) for binomial distributions. The factors *cutting date* and *temperature* were included as fixed factors in the first models. As there was no effect of the *temperature*, the final models only included cutting date or seed color fixed factors. We added an error term for repeated measures to the models to account for variation within each *sampling location*. Furthermore, we added a general linear hypothesis and multiple comparisons (glht) to determine significant differences between groups.

To identify seed traits associated with germination success, we checked for correlation of seed traits with the factor *cutting date* using Pearson's R^2. This was the case for *seed size*, *seed color*, and *proportion of hard seeds*. We then fitted models with these traits as fixed factors (both in combinations and as single-factor models) and *sampling*

location as random factor. To choose the best seed traits or trait combination to explain germination success of *L. polyphyllus*, we compared these models via AIC and pairwise model ANOVA. To assess model quality, we calculated Nagakawa and Schielzeth's R^2 for linear mixed-effect models (Nakagawa and Schielzeth 2013). We visually checked for normality of residuals and homogeneity of variances using diagnostic plots (Zuur et al. 2010). Mixed-effect models were carried out using the 'lme4' (Bates et al. 2015) and 'lmerTest' (Kuznetsova et al. 2017) packages, post-hoc-tests were calculated using the 'multcomp' package (Hothorn et al. 2008), graphs were created using the 'ggplot2' package (Wickham 2016) in R (R Core Team 2016).

Results

During the sampling period, seed color became darker (changing from green via dark green and brown, to black) and the proportion of hard seeds increased gradually. Mean seed size ranged from 3.9 mm (date six, August 11th) to 6.4 mm (date three, July 21st). It increased during the first three weeks of cutting and then decreased thereafter as seeds became drier. Seed color and the proportion of hard seeds were correlated, as hard seeds usually were darker than soft seeds. There were no differences in the total germination percentages between different *sampling locations*, although the germination peaks shifted by up to two weeks between different locations.

In climate chambers, 16.3% of all collected lupine seeds germinated (Fig. 1a, b). Germination percentage was lowest after the first cutting date (July 7th, 8.6%) and increased until the third date (July 21st) where it peaked at 26% (Fig. 1a). Afterwards, we observed a significant decrease from week three (July 21st) to four (July 28th; to 13.4%; Table 1). Mean germination time was 114 days and varied from 3 days to 303 days in climate chambers (Fig. 1c, d), with seeds collected on the first date having the longest mean germination time (141 d; Fig. 2d). Mean germination time decreased until week three (98 d), then increased again and had its overall minimum in week six (74 d). Synchrony of germination was quite low with an average of 0.08 over all cutting dates (Fig. 1e, f).

In the common garden, 51.7% of seeds germinated and mean germination time was 153.6 days (Fig. 2). Thus, seeds in the common garden germinated to a higher degree compared to seeds in climate chambers, but slower. Germination percentages in the common garden were lowest in seeds sampled during the first two weeks (17.0% on July 7th and 30.6% on 14th), reached the highest level in week three (63.2% on July 21st) and stayed high afterwards (Fig. 2a, Table 1). In the common garden, mean germination time was similar for all cutting dates and averaged 153.6 days. Synchrony of germination in the common garden was quite low with an average of 0.12 over all treatments and on all cutting dates (Fig. 2e, f).

There were significant differences in germination percentages between seeds of different color (Figs 1b, 2b, Table 2). In climate chambers, dark green seeds showed the highest germination while in the common garden, germination percentages increased steadily as seeds darkened (Figs 1b, 2b). In climate chambers, germination percentage peaked when 60% of collected seeds were hard and decreased when the amount of hard seeds was lower

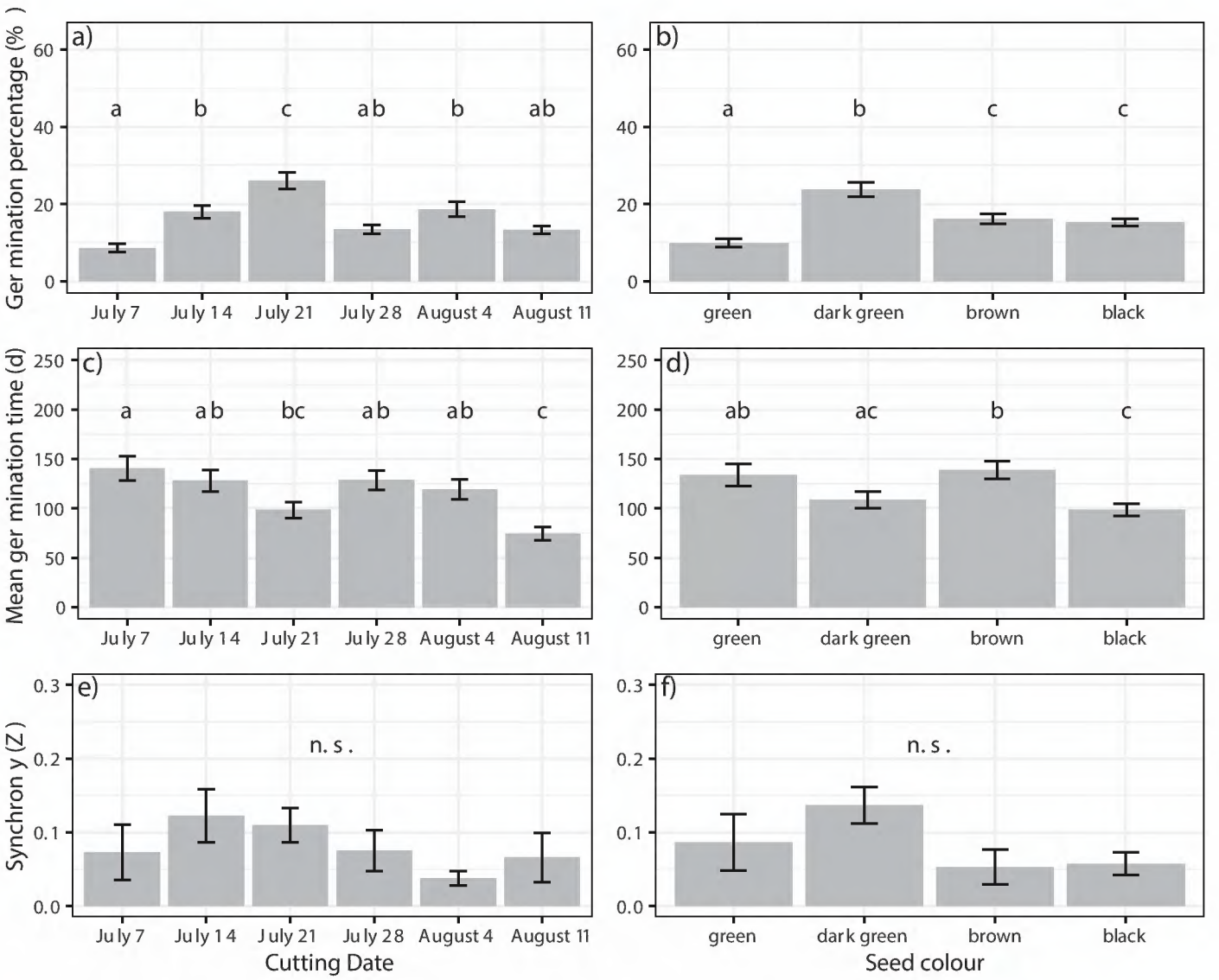

Figure 1. The effect of the factors cutting date (weekly from July 7th to August 11th) and seed color on germination percentage (**a, b**), mean germination time (**c, d**), and synchrony of germination (**e, f**) in seeds stored in climate chambers averaged over the two temperature regimes. Bars show mean values ± standard errors.

Table 1. Differences in germination percentages of *L. polyphyllus* seeds between six cutting dates assessed in two germination experiments (climate chamber and common garden). Differences were assessed using mixed effect models for each experiment separately with sampling location as random factor (formula: Germination percentage ~ Cutting Date + (1|Sampling location).

Climate chamber	**n = 300**	**$R^2_{marginal}$ = 0.20**	**$R^2_{conditional}$ = 0.25**		
	Estimate	**Std. Error**	**Df**	**t Value**	**p Value**
Date 1 (July 7; Intercept)	8.64	1.86	22.56	4.65	< 0.001
Date 2 (July 14)	9.36	2.11	295	4.45	< 0.001
Date 3 (July 21)	17.36	2.11	295	8.25	< 0.001
Date 4 (July 28)	4.8	2.11	295	2.28	0.023
Date 5 (August 4)	10	2.11	295	4.75	< 0.001
Date 6 (August 11)	4.64	2.11	295	2.20	0.028
Common garden	**n = 150**	**$R^2_{marginal}$ = 0.63**	**$R^2_{conditional}$ = 0.71**		
	Estimate	**Std. Error**	**Df**	**t Value**	**p Value**
Date 1 (July 7; Intercept)	16.96	3.88	14.21	4.37	< 0.001
Date 2 (July 14)	13.6	3.86	145	3.52	< 0.001
Date 3 (July 21)	46.24	3.86	145	11.99	< 0.001
Date 4 (July 28)	47.68	3.86	145	12.37	< 0.001
Date 5 (August 4)	52	3.86	145	13.49	< 0.001
Date 6 (August 11)	48.8	3.86	145	12.66	< 0.001

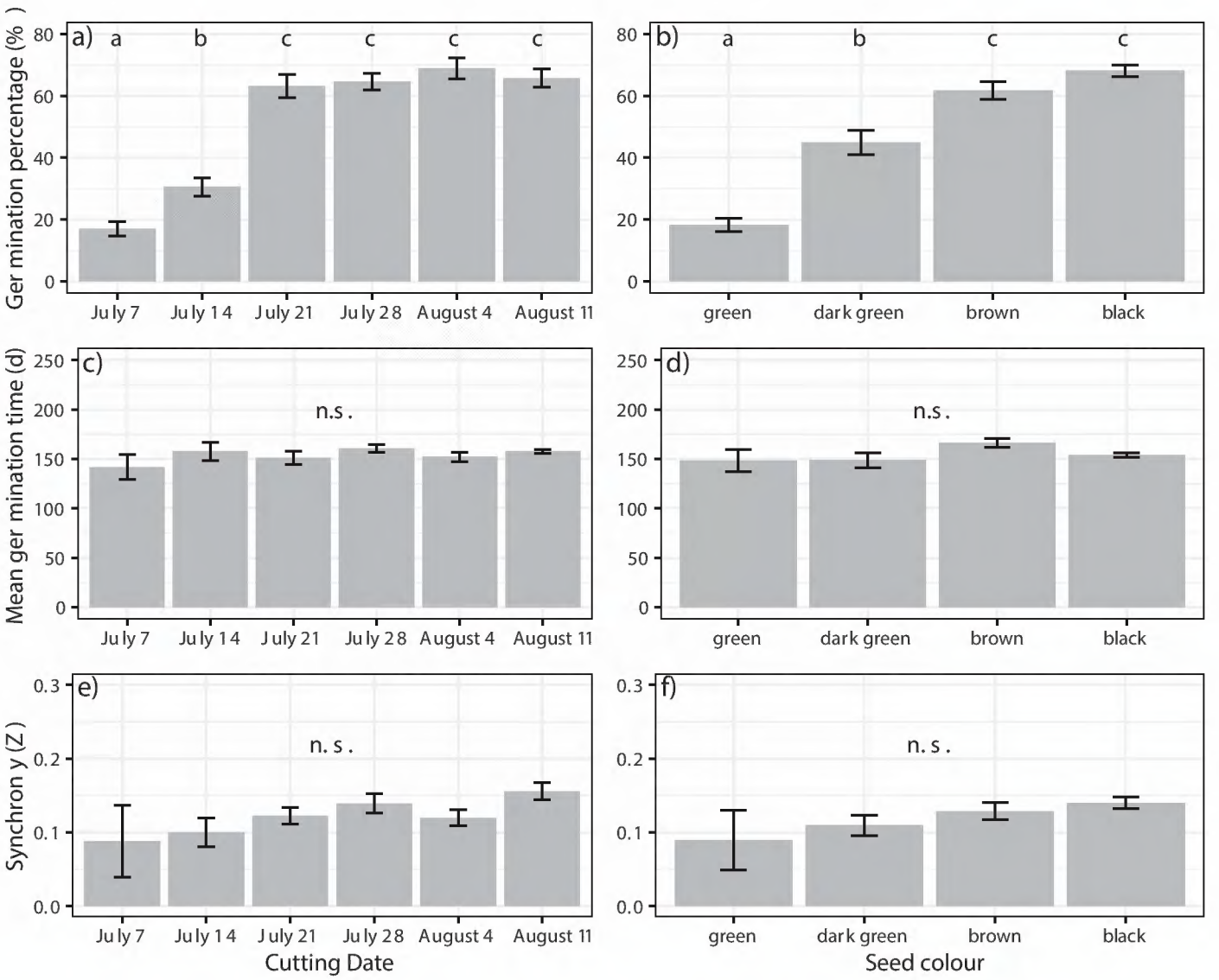

Figure 2. The effect of the factors cutting date (weekly from July 7th to August 11th) and seed color on germination percentage (**a, b**), mean germination time (**c, d**), and synchrony of germination (**e, f**) in seeds stored under ambient weather conditions. Bars show mean values ± standard errors.

Table 2. Differences in germination percentages of *L. polyphyllus* seeds between four seed colors (median seed color per sample with four levels: green, dark green, brown, and black) assessed in two germination experiments (climate chamber and common garden). Differences were assessed using mixed effect models for each experiment separately with sampling location as random factor (formula: Germination percentage ~ Seed color + (1|Sampling location).

Climate chamber	**n = 300**	**$R^2_{marginal}$ = 0.15**	**$R^2_{conditional}$ = 0.22**		
	Estimate	**Std. Error**	**Df**	**t Value**	**p Value**
Green (Intercept)	9.45	1.88	16.2	5.03	< 0.001
Dark green	14.34	1.94	298.48	7.4	< 0.001
Brown	7.71	2.12	299.21	3.64	< 0.001
Black	5.64	1.74	297.85	3.25	< 0.01
Common garden	**n = 150**	**$R^2_{marginal}$ = 0.58**	**$R^2_{conditional}$ = 0.65**		
	Estimate	**Std. Error**	**Df**	**t Value**	**p Value**
Green (Intercept)	17.29	4.11	12.24	4.21	< 0.01
Dark green	27.78	3.82	146.4	7.27	< 0.001
Brown	47.13	4.18	146.84	11.27	< 0.001
Black	50.14	3.42	146.09	14.65	< 0.001

or higher while in the common garden, germination percentage increased continuously with the amount of hard seeds. In both experiments, seeds of different color had relatively similar germination times with black (99 d) and dark green (109 d) seeds germinating most rapidly in climate chambers (Fig. 1d). In the common garden, there were no significant differences in mean germination time between seeds of different colors (Fig. 2d).

While in climate chambers, germination peaked early and decreased afterwards (Fig. 3), two peaks (in autumn and spring) characterized germination in the common garden (Fig. 4). There were no significant differences between colors in climate chamber, while synchrony in the common garden increased slightly with the increase in the percentage of hard seeds.

Germination percentage (in both experiments) and mean germination time (only in climate chambers) responded significantly to *cutting date*, while there was no effect of the different *temperature* regimes. For germination percentage and mean germination time, the best explanatory models (see Suppl. material 1: Model Tables) each contained solely one fixed factor, mainly due to high correlations between explaining factors. Germination percentage in climate chambers was best explained by seed color and showed highly significant differences between colors ($R^2 = 0.15$). Germination percentage in the common garden was well explained by both seed color ($R^2 = 0.58$) and proportion of hard seeds. For mean germination time, the best explaining factors were either color or proportion of hard seeds, while both models performed poorly overall. Synchrony was not affected significantly by any factor and there was no model of significant explanatory value.

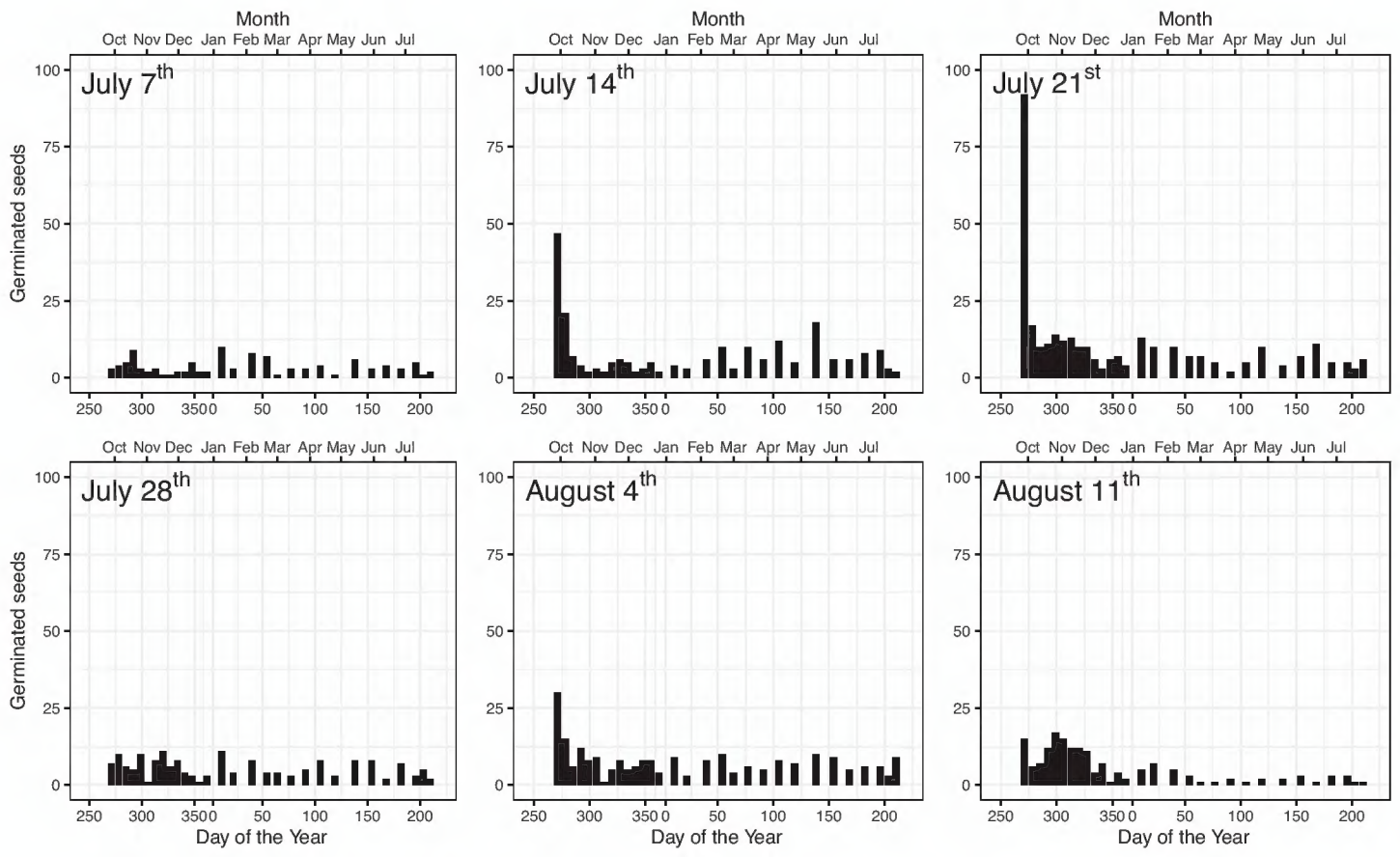

Figure 3. Germination patterns of *L. polyphyllus* in climate chambers conditions (15/5 °C and 20/10 °C diurnally fluctuating temperatures) sampled weekly on six cutting dates (July 7th to August 11th) after seed set.

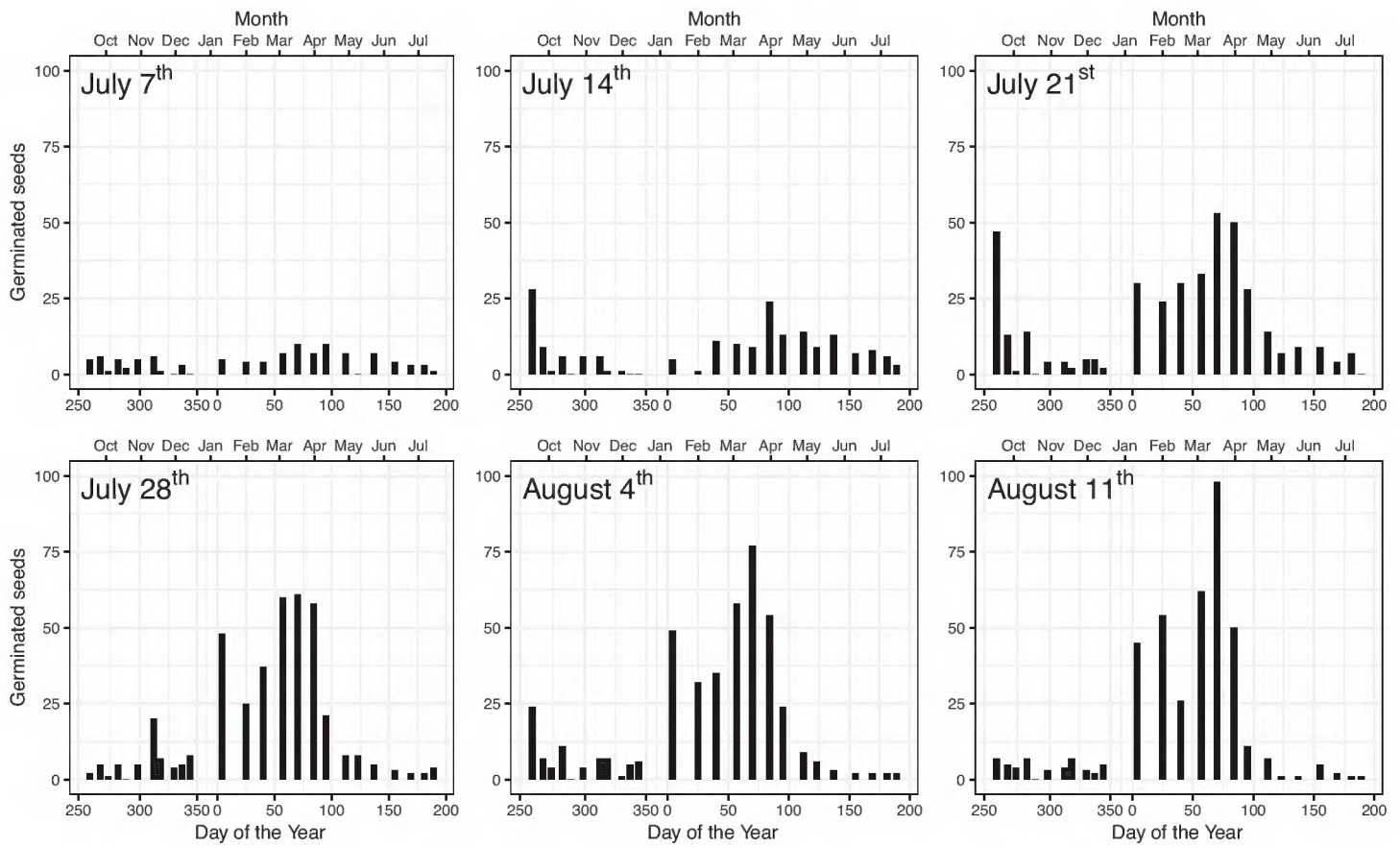

Figure 4. Germination patterns of *L. polyphyllus* under ambient weather conditions sampled weekly on six cutting dates (July 7th to August 11th) after seed set.

Discussion

The germination patterns of the invasive legume *L. polyphyllus* differed between different cutting dates, partially confirming our first hypothesis. Seeds collected early, while being green and soft, germinated to a lower degree and more slowly compared to seeds collected later. While seeds of early-cut *L. polyphyllus* plants germinated in autumn, seeds of late-cut plants were more prone to germinate in spring. This relationship may be associated with their progression through different phases of seed development. During morphogenesis the embryo develops, then during maturation, storage compounds are synthesized in the growing endosperm and thereafter, seeds may go through a phase of desiccation, in which they dry and eventually enter dormancy (Angelovici et al. 2010). Consequently, the different cutting dates of our experiment covered the phases of maturation and desiccation. Until late July, *L. polyphyllus* seeds were in the phase of maturation. Afterwards (end-July to mid-August), seeds were in the desiccation phase. Although dormancy per se was not tested in our study, the observed germination patterns and differences between climate chambers and the common garden strongly indicate that seeds from late cut *L. polyphyllus* plants expressed dormancy, which is also supported by our observation that seeds decreased in size and became harder. Physical dormancy is common in legumes (Russi et al. 1992a), but whether an individual plant produces dormant seeds at a given point in time depends on a variety of factors, such as temperature and moisture conditions during seed ripening (Masaka and Yamada 2009; Bolingue et al. 2010; D'hondt et al. 2010). Thus, the expression of dormancy can vary strongly in legume seeds, even within plants of the

same population (D'hondt et al. 2010), which may consequently lead to asynchronous germination patterns.

Despite pronounced peaks of germination in autumn and spring, germination of *L. polyphyllus* seeds was highly asynchronous. In both experiments and under all cutting dates, some seeds germinated over the whole duration of the experiments, over 300 days. The timing of germination determines which environmental conditions the seedling will experience and may influence plant characteristics, such as growth and reproduction (Donohue 2002; Casas et al. 2012). The timing of germination itself may be influenced by plant life-history traits, e.g. the phenology of flowering, seed maturation, and seed dispersal (Galloway 2001; Donohue 2002). Variations in germination depending on the time of seed collection have been observed by other authors (e.g., Greipsson and El-Mayas 2003; Samarah 2005; El-Keblawy and Al-Rawai 2006; Brobäck 2015), but there is little information on the long-term germination patterns of species and seasonal effects that are associated with this factor. In invasive species, asynchronous germination can lead to the exploitation of open germination niches throughout the year, which might contribute to their invasion success (Wolkovich and Cleland 2011; Gioria et al. 2016). In the case of *L. polyphyllus*, this effect may be amplified by its high seed production (Volz 2003), its long-lasting flowering, by its ability to resprout and produce seeds after early cutting (Brobäck 2015), and by the observation that the ballistic seed dispersal of the species takes place over many weeks if stands are left untouched (Klinger et al., unpublished data). The observed germination patterns of *L. polyphyllus* thus suggest a bet-hedging strategy (Cohen 1966), which may partly explain its invasion success and its capability to colonize many different habitats.

Our second hypothesis can be verified, as seed color and the percentage of hard seeds were good predictors of germination percentage and give information on the germination patterns that can be expected. Soft and green seeds germinated to the lowest degree and in autumn. However, germination percentages of these seed batches were relatively high, given their early developmental phase. High germination rates in immature seeds have been found in some legumes, e.g., in *Lotus* and *Scorpiurus* (Cristaudo et al. 2008), and *Vicia* (Samarah 2005), but germination failed in others, such as in green seeds of *Lupinus nootkatensis* (Greipsson and El-Mayas 2003). Black and hard seeds germinated to a high degree and in spring. In temperate climates, seedlings germinating in autumn face harsh environmental conditions during winter combined with low competition, while spring germination is associated with more favorable environmental conditions, but higher competition (Masuda and Washitani 1992). Since soft and green seeds mostly germinated in autumn, the winter survival of the emerging seedlings may be low, as *L. polyphyllus* seedlings seem to be sensitive to freezing and showed high mortality when exposed to –10 °C (Arfin-Khan et al. 2018). Furthermore, unripe seeds of roadside *L. polyphyllus* stands in Sweden were prone to mold infection that led to very low germination rates (Brobäck 2015). The last cutting date represents the state in which seeds are shed by the plant. Both ballistic seed dispersal as well as the expression of physical dormancy go along with the drying of the pods and the seed coat. Black and hard seeds are more prone to germinate in spring and may thus have higher survival rates compared to green seeds. Furthermore, as *L. polyphyllus* follows a c-strategy (Grime et al. 1988), it may be able to cope with higher competition

in spring, especially in habitats with weak competitors, such as semi-natural grasslands. Additionally, water impermeable/hard seeds are more prone to being carried over into the seed bank (Russi et al. 1992b) or dispersed via endozoochory (Otte et al. 2002; D'hondt and Hoffmann 2011). Although *L. polyphyllus* may not have invaded the seed bank of meadows in our study region yet (Ludewig et al., unpublished data), a carry-over of seeds should be avoided, as it makes invasive species management lengthier and more expensive. Consequently, managers should target plants that still have green and soft seeds, which can be considered less problematic despite germination percentages being relatively high.

Germination patterns differed between climate chambers and the common garden, particularly after seeds darkened and became harder. Overall, germination percentages in the climate chamber experiment (ca. 16%) were similar to the emergence rates found by Sõber and Ramula (2013) (21.5%), but relatively low compared to other studies on *L. polyphyllus* (Elliott et al. 2011; Arfin-Khan et al. 2018; over 60%). We suggest that this is at least partly due to the fact that seeds were not scarified and that dormancy was probably not broken by imbibition in the climate chamber experiment. This is also supported by the results of the common garden experiment, in which germination percentages were considerably higher than under laboratory conditions, mainly due to a second germination peak in spring after winter-stratification *in situ*. However, germination of *L. polyphyllus* only slightly increased when seeds were pre-treated by cold in another study (Elliott et al. 2011). Our results show that, while laboratory experiments give valuable information on the environmental factors influencing germination, the germination patterns observed under artificial conditions may diverge from germination dynamics under ambient weather conditions (Hölzel and Otte 2004). A better understanding of invasive species germination under natural conditions is necessary, as it can potentially reveal windows of opportunity for invasive species management. We thus recommend to complement germination experiments in climate chambers with common garden or field experiments.

Conclusions

Seeds of *L. polyphyllus* are capable of after-ripening and germinating even if plants are cut while most seeds are still green and soft. Germination capability increased strongly during the first weeks after seed set with a maximum when most seeds were brown to black and not fully hardened. Therefore, *L. polyphyllus* stands should be cut before seed set, if possible. If this is not feasible due to different limitations, we recommend cutting while plants carry green and soft seeds. When stands with black and hard seeds are cut, the plant material should be removed immediately to reduce propagule pressure on site.

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Supplementary material 1

Model Tables

Authors: Yves P. Klinger, Rolf Lutz Eckstein, David Horlemann, Annette Otte, Kristin Ludewig

Data type: table xlsx-file

Explanation note: Model summary tables.

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